The specification has been further amended to recite the current address of the American Type Culture Collection.

Amendments to the Claims

Claims 16, 17, 21, 22, 25, 26 and 29-40, drawn to non-elected inventions (see, Paper No. 7 at page 2, Paragraph No. 1), have been cancelled without prejudice. Claims 1, 4-9, 11-15, 18-20, 23, 24, 27, 28 and 41-45 have been amended, and new Claims 46-56 have been added. Claims 1-9, 11-15, 18-20, 23, 24, 27, 28 and 41-56 are pending.

Support for amended Claim 5 is found, for example, at page 12, lines 12-21, Figures 7 and 9, and pages 33 to 42 (Example 1).

Support for amended Claim 7 is found, for example, at page 17, lines 15-21.

Support for amended Claim 8 is found, for example, at page 15, lines 6-10.

Support for amended Claim 11 is found, for example, in Figure 5.

Support for amended Claim 12 is found, for example, in Figure 6.

Support for amended Claim 13 is found, for example, at page 15, lines 6-10.

Support for amended Claims 14 and 15 is found, for example, at page 15, lines 6-10, and in Figure 5.

Support for amended Claims 19 and 20 is found, for example, at page 18, line 6 *et seq.*, and in Figure 6.

Support for amended Claims 23, 24, 27 and 28 is found, for example, at page 18, line 6 *et seq.*

Support for amended Claims 41-43 and 45 is found, for example, at page 11, line 20 *et seq.*

Claims 46-56 are new. Support for the claims is found throughout the application, for example, at page 14, line 8 *et seq.*, Figures 7 and 9, and pages 25-28.

The amendments to the claims find support in specification and claims as filed. Therefore, this Amendment adds no new matter.

Applicants thank the Examiner for conducting an interview on August 31, 2000, with Applicants' representatives and Lee R. Brettman, M.D., and for his comments in the Interview Summary (Paper 26) regarding obviating the rejection under 35 U.S.C. § 103 (the only remaining rejection) in view of declarations indicating that the Act-1 hybridoma cell line was not freely publicly available at the time the invention was made. Declarations under 37 C.F.R. § 1.132 of Robert B. Colvin, M.D., Walter Newman, Ph.D. and Barry Coghlin are being filed concurrently with this Preliminary Amendment. Favorable consideration is respectfully requested.

Rejection of Claims 1-9, 11-15, 18-20, 23, 24, 27 and 28 Under 35 U.S.C. § 103(a)

Claims 1-9, 11-15, 18-20, 23, 24, 27 and 28 are rejected under 35 U.S.C. § 103(a) as being obvious over Queen *et al.* (U.S. Patent No. 5,530,101) in view of Lazarovits *et al.* (*J. Immunol.*, *151*:6482-6489 (1993)) and in further evidence by the "Information for Contributors" of volume 151 of the *Journal of Immunology*.

The rejection is based on a presumption that the Act-1 hybridoma was publicly available, and that, using a variety of procedures known in the art, the person of ordinary skill would have a reasonable expectation of success of cloning the rearranged variable regions and of producing humanized antibodies, heavy chains, light chains and fragments thereof in accordance with the claims. Relying as it does upon cloning of rearranged variable regions encoding the murine Act-1 antibody, the rejection hinges upon the public availability of the Act-1 hybridoma.

As stated by Applicants in the record, the Act-1 hybridoma cell line was produced by Dr. Andrew I. Lazarovits while he was working as a research fellow in the laboratory of Dr. Robert B. Colvin at Massachusetts General Hospital. LeukoSite, Inc. (now Millennium Pharmaceuticals, Inc.) Assignee of the subject application, obtained the Act-1 hybridoma subject to a License Agreement with The General Hospital Corporation, employer of Drs. Lazarovits and Colvin at the time the Act-1 antibody was made (Paper No. 24 at page 12, lines 19-24). Dr. Andrew I. Lazarovits died on January 29, 1999.

The Declarations of Drs. Colvin and Newman and Mr. Coghlin, filed concurrently herewith, support these statements and provide evidence that the Act-1 hybridoma cell line was not freely publicly available prior to the making of the invention or the filing date of the application. For example, in his declaration, Dr. Colvin describes circumstances under which samples of the Act-1 hybridoma cell line were provided to others from his laboratory at Massachusetts General Hospital. In particular, Dr. Colvin describes the circumstances under which a sample of the Act-1 hybridoma was provided to Becton Dickinson Advanced Cellular Biology for evaluation of the Act-1 antibody as a potential diagnostic agent (Colvin Declaration at Paragraphs 6 and 7), and the circumstances under which a sample of the Act-1 hybridoma was given to LeukoSite, Inc (Colvin Declaration at Paragraph 8). Dr. Colvin also states that samples of the Act-1 hybridoma cell line have not been provided from his laboratory to any others (Colvin Declaration at Paragraph 9). Dr. Colvin further states that he remained in contact with Dr. Lazarovits until Dr. Lazarovits' death, and that it is his understanding that Dr. Lazarovits did not distribute the Act-1 hybridoma cell line from his laboratory (Colvin Declaration at Paragraph 10).

The Declarations of Dr. Newman and Mr. Coghlin corroborate Dr. Colvin's statement regarding his understanding that Dr. Lazarovits did not distribute the Act-1 hybridoma cell line and provide further evidence that the Act-1 hybridoma cell line was not freely publicly available prior to the making of the invention or filing date of the subject application.

Since the Act-1 hybridoma cell line was not freely publicly available at the time the invention was made or the application was filed, the rearranged variable region genes encoding the variable regions, including the complementarity determining regions (CDRs), of the Act-1 monoclonal antibody were not available to the person of ordinary skill in the art at those times. Absent such availability, the amino acid sequences of the variable regions and CDRs of the Act-1 monoclonal antibody and the claimed humanized antibodies are nonobvious. Favorable consideration of the Declarations and withdrawal of the rejection in view of same is respectfully requested.

Request for Rejoinder Pursuant to M.P.E.P. § 821.04

Claims 41-45 and new Claims 46-52 and 54-56 are drawn to "methods of use" of a humanized antibody or antigen-binding fragment. During the interview conducted on August 31, 2000, the Examiner indicated that the "method of use" claims will be rejoined and allowed if the product claims are deemed allowable, and evidence of clinical efficacy is provided. The product claims (Claims 1-9, 11-16, 19, 20, 23, 24, 27, 28 and 53) are believed to be allowable in view of the Declarations of Dr. Colvin, Dr. Newman and Mr. Coghlin. Evidence of therapeutic efficacy is provided by Feagan S.B. *et al.*, *Gastroenterology*, *118*(4):A874 (2000), Reference AU5 in the Information Disclosure Statement being filed concurrently herewith.

Feagan *et al.* describe the results of a clinical study in which an antibody of the present invention (LDP-02, see Specification, Example 4 at page 90 *et seq.*) was administered to patients with moderately severe ulcerative colitis. Effectiveness measurements were collected during the study. Feagan *et al.* report that "40% of Group 3 patients [single administration of LDP-02 at 0.5 mg/Kg IV] had a complete endoscopic (modified Baron's score = 0) and clinical (MCS = 0) remission" (Feagan *et al.* at lines 37-38 of the abstract).

In view of the efficacious treatment of patients with moderately severe ulcerative colitis reported by Feagan *et al.*, Applicants respectfully request that Claims 41-45 and new Claims 46-52 and 54-56 be rejoined, pursuant to U.S. Patent Office practice (M.P.E.P. § 821.04), if product claims are found to be allowable.

Information Disclosure Statement

An Information Disclosure Statement (IDS) is being filed concurrently herewith. Acknowledgment of consideration of the information contained therein is respectfully requested in the next Office Communication.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 39, line 27 to page 40, line 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

For transient expression of the chimeric antibody, 20 μg of pEE12mhLHchi was transfected into COS-7 cells (American Type Culture Collection, [12301 Parklawn Drive, Rockville, MD, 20852] 10801 University Boulevard, Manassas, VA 20110-2209) by electroporation as follows. COS-7 cells growing in log phase were harvested from tissue culture flasks by treatment with trypsin-EDTA. The cells were washed once in Phosphate Buffered Saline (PBS), once with Hank's Balanced Salts Solution (HBSS), and resusended at a concentration of 1.5×10^7 cells per ml of HBSS. 1.2×10^7 cells in 0.8 ml HBSS was mixed with 20 μg of the plasmid DNA and incubated for 10 minutes at room temperature. The DNA/cell mixture was then transferred to a 0.4 cm electroporation cuvette and current applied at 250 V, 960 μF with a Bio-Rad GenePulser. After a 10 minute post-electroporation incubation at room temperature, the cells were transferred to 20 mls of culture medium (Dulbecco's Modified Eagle's Medium (DMEM) plus 10% FCS) and cultured in a 162 cm^2 tissue culture flask (Costar). After 5 days, the cell culture supernatant was harvested and tested for the ability to stain HuT 78 cells which express the α4β7 integrin. HuT 78 cells (a human T cell lymphoma line) are available from the American Type Culture Collection, [12301 Parklawn Drive, Rockville, MD, 20852] 10801 University Boulevard, Manassas, VA 20110-2209, Accession No. ATCC TIB 161.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Claims 16, 17, 21, 22, 25, 26 and 29-40 have been cancelled and New Claims 46-56 have been added.

1. (Amended Five Times) A humanized immunoglobulin or antigen-binding fragment thereof having binding specificity for α4β7 integrin, said immunoglobulin or fragment comprising an [antigen binding] antigen-binding region of nonhuman origin and at least a portion of an immunoglobulin of human origin, said [antigen binding] antigen-binding region comprising at least one of three complementarity determining regions (CDR1, CDR2 and CDR3) of a light chain variable region and at least one of three complementarity determining regions (CDR1, CDR2 and CDR3) of a heavy chain variable region of the amino acid sequence set forth below such that the antibody specifically binds to the α4β7 integrin:

light chain: CDR1 amino acids 44-59 of SEQ ID NO: 12
CDR2 amino acids 75-81 of SEQ ID NO: 12
CDR3 amino acids 114-122 of SEQ ID NO: 12
heavy chain: CDR1 amino acids 50-54 of SEQ ID NO: 15
CDR2 amino acids 69-85 of SEQ ID NO: 15
CDR3 amino acids 118-129 of SEQ ID NO: 15.

4. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 2 wherein the [antigen binding] antigen-binding region is of rodent origin.

5. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 2 [wherein the antigen binding region is derived from Act-1 monoclonal antibody] comprising:

an immunoglobulin light chain variable region comprising the amino acid sequence of amino acids 21-132 of SEQ ID NO:12; and

an immunoglobulin heavy chain variable region comprising the amino acid sequence of amino acids 20-140 of SEQ ID NO:15.

6. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 1 wherein [the antigen binding region comprises a complementarity determining region of rodent origin, and] the portion of an immunoglobulin of human origin is derived from a human framework region.

7. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 6, wherein [the complementarity determining region is derived from] said immunoglobulin or fragment can compete with Act-1 monoclonal antibody (ATCC Accession No. PTA-3663) for binding to α4β7 integrin.

8. (Amended Five Times) A humanized immunoglobulin or antigen-binding fragment thereof having binding specificity for α4β7 integrin comprising a heavy chain and a light chain,

the light chain comprising complementarity determining regions derived from an antibody of nonhuman origin which binds α4β7 and a framework region derived from a light chain variable region of human origin, wherein each of said complementarity determining regions (CDR1, CDR2 and CDR3) comprises the amino acid sequence set forth below [such that the immunoglobulin or fragment specifically binds to the α4β7 integrin]:

light chain: CDR1 amino acids 44-59 of SEQ ID NO: 12
CDR2 amino acids 75-81 of SEQ ID NO: 12
CDR3 amino acids 114-122 of SEQ ID NO: 12; and

the heavy chain comprising complementarity determining regions derived from an antibody of nonhuman origin which binds α4β7 and a framework region derived from a heavy chain variable region of human origin, wherein each of said complementarity determining regions (CDR1, CDR2 and CDR3) comprises the amino acid sequence set forth below [such that the immunoglobulin or fragment specifically binds to the α4β7 integrin]:

heavy chain: CDR1 amino acids 50-54 of SEQ ID NO: 15
CDR2 amino acids 69-85 of SEQ ID NO: 15
CDR3 amino acids 118-129 of SEQ ID NO: 15.

9. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 8 wherein said immunoglobulin can compete with murine Act-1 monoclonal antibody (ATCC Accession No. PTA-3663) for binding to $\alpha 4\beta 7$.

11. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 8 wherein [the] said light chain variable region of human origin is the light chain variable region of the human GM607'CL antibody (SEQ ID NO: 8).

12. (Twice Amended) The humanized immunoglobulin or antigen-binding fragment of Claim 8 wherein [the] said heavy chain variable region of human origin is the heavy chain variable region of the human 21/28'CL antibody (SEQ ID NO: 10).

13. (Amended Five Times) A humanized immunoglobulin light chain or antigen-binding portion [fragment] thereof comprising complementarity determining regions (CDR1, CDR2 and CDR3) of the light chain of murine Act-1 antibody (ATCC Accession No. PTA-3663), and a framework region derived from a [human] light chain [framework] variable region of human origin, said complementarity determining regions comprising the amino acid sequences set forth below such that an antibody or antigen-binding fragment thereof comprising said light chain or [fragment] antigen-binding portion thereof specifically binds to the $\alpha 4\beta 7$ integrin:

light chain: CDR1 amino acids 44-59 of SEQ ID NO: 12
CDR2 amino acids 75-81 of SEQ ID NO: 12
CDR3 amino acids 114-122 of SEQ ID NO: 12.

14. (Twice Amended) The humanized immunoglobulin light chain or antigen-binding portion [fragment] thereof of Claim 13 wherein the human framework region is derived from the light chain variable region of the human GM607'CL antibody (SEQ ID NO: 8).

15. (Twice Amended) The humanized immunoglobulin light chain or antigen-binding portion [fragment] thereof of Claim 14 comprising the variable region of SEQ ID NO:21.

18. (Amended Five Times) A humanized immunoglobulin heavy chain or antigen-binding portion [fragment] thereof comprising complementarity determining regions (CDR1, CDR2 and CDR3) of the heavy chain of the murine Act-1 antibody (ATCC Accession No. PTA-3663), and a framework region derived from a [human] heavy chain [framework] variable region of human origin, said complementarity determining regions comprising the amino acid sequences set forth below such that an antibody or antigen-binding fragment thereof comprising said heavy chain or antigen-binding portion [fragment] thereof specifically binds to the α4β7 integrin:

heavy chain: CDR1 amino acids 50-54 of SEQ ID NO: 15
CDR2 amino acids 69-85 of SEQ ID NO: 15
CDR3 amino acids 118-129 of SEQ ID NO: 15.

19. (Twice Amended) The humanized immunoglobulin heavy chain or antigen-binding portion [fragment] thereof of Claim 18 wherein the human framework region is derived from the heavy chain variable region of the human 21/28'CL antibody (SEQ ID NO: 10).

20. (Twice Amended) The humanized immunoglobulin heavy chain or antigen-binding portion [fragment] thereof of Claim 19 comprising the variable region of SEQ ID NO:19.

23. (Amended Three Times) A humanized immunoglobulin light chain, the amino acid sequence of said light chain comprising at least [a functional] an antigen-binding portion of the light chain variable region amino acid sequence shown in Figure 7 (amino acids 21-132 of SEQ ID NO:12)[, wherein said functional portion has binding specificity for α4β7 integrin].

24. (Twice Amended) [A] The humanized immunoglobulin light chain of Claim 23, wherein said amino acid sequence of said light chain comprises the signal peptide sequence shown in Figure 7 (amino acids 1-20 of SEQ ID NO:12) and at least [a functional] an antigen-binding portion of the light chain variable region amino acid sequence shown in Figure 7 (amino acids 21-132 of SEQ ID NO:12).

27. (Amended Three Times) A humanized immunoglobulin heavy chain, the amino acid sequence of said heavy chain comprising at least [a functional] an antigen-binding portion of the heavy chain variable region amino acid sequence shown in Figure 9 (amino acids 20-140 of SEQ ID NO:15)[, wherein said functional portion has binding specificity for α4β7 integrin].

28. (Twice Amended) [A] The humanized immunoglobulin heavy chain of Claim 27, wherein said amino acid sequence of said heavy chain comprises the signal peptide sequence shown in Figure 9 (amino acids 1-19 of SEQ ID NO:15) and at least [a functional] an antigen-binding portion of the heavy chain variable region amino acid sequence shown in Figure 9 (amino acids 20-140 of SEQ ID NO:15).

41. (Amended) A method of inhibiting the interaction of a first cell bearing α4β7 with a second cell bearing a ligand thereof, comprising contacting said first cell with an effective amount of a humanized immunoglobulin or antigen-binding fragment of Claim 1.

42. (Amended) A method of inhibiting leukocyte infiltration of mucosal tissue, comprising administering to a patient an effective amount of a humanized immunoglobulin or antigen-binding fragment of Claim 1.

43. (Amended) A method of therapy of a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1, comprising administering to a patient an effective amount of a humanized immunoglobulin or antigen-binding fragment of Claim 1.

44. (Amended) The method of Claim 43, wherein the disease is a disease associated with leukocyte infiltration of tissues as a result of binding of leukocytes to gut-associated endothelium expressing the molecule MAdCAM-1.

45. (Amended) A method for treating inflammatory bowel disease in a patient, comprising administering to the patient an effective amount of a humanized immunoglobulin or antigen-binding fragment of Claim 1.